BIO-CHEMICAL AND THERAPEUTICAL STUDIES ON TRYPANOSOMIASIS

BY

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(*Received for publication* 15 *August*, 1909)

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The following is a detailed report of our observations on experimental Trypanosomiasis, the treatment of infections with different pathogenic trypanosomes, and the mechanism of the therapeutical action of various trypanocidal compounds. The experiments were carried out in this laboratory during the last two years.

Our thanks are due to Sir Alfred Jones, through whose generosity we were able to extend our experiments upon monkeys. Further, we wish to express our indebtedness to Messrs. Burroughs, Wellcome and Co., who supplied us with a great number of trypanocidal drugs and supported the work very materially. We also have to express our thanks to Professor Ehrlich for the supply of different compounds prepared in his laboratory, and to Messrs. Bayer and Co. who put at our disposal a number of aniline dyes for our experimental work.

INTRODUCTION

The ease with which infection caused by the different pathogenic trypanosomes can be transmitted from animal to animal, and especially the fatality of such infection in small laboratory animals, has attracted the attention of many workers.

The action of various compounds which are chemically allied has been studied with the object of finding an absolute cure for different trypanosome infections. This would obviously be of the utmost economic importance in different parts of the tropics.

Efforts in this direction have led to great progress in our knowledge of the biology of trypanosomes; they have thrown much light on protozoal diseases in general; they have advanced our experience in experimental chemotherapeutics, and have opened up a new and large field for original investigation.

The large number of drugs of specific trypanocidal character can be classified into three distinct groups. To the first group belong compounds containing as their active principle arsenic in an inorganic form, such as Sodium arsenate; to the second, those in which the arsenic molecule is masked by different organic radicals, such as the aniline nucleus, in Atoxyl and numerous allied compounds. To this second group also belong the different colouring matters of the diazo type: trypanred, trypanblue, and derivatives of the triphenylmethan type, such as malachite green, parafuchsin and tryparosan. The third group consists of Antimony in the form of sodium antimonyl tartrate and in the form of the three isomeric arylstibinic acids.

ORGANIC ARSENIC COMPOUNDS

A. *Atoxyl.*

Shortly after the discovery of the therapeutic value of Atoxyl in experimental Trypanosomiasis, this drug was extensively studied. It has been applied in the treatment of Sleeping Sickness and natural trypanosome infection in cattle, with very varying results. The mechanism of its action has been carefully studied, and Atoxyl may be regarded at present as one of the best known drugs.

Thomas and Breinl* first introduced Atoxyl into the treatment of

* (*a*) Thomas. British Medical Journal, May, 1905.

(*b*) Thomas and Breinl. Liverpool School of Tropical Medicine, Memoir XVI, 1905.

experimental Trypanosomiasis with a view to administering arsenic in larger doses and in a less toxic form than sodium arsenate, which was already known to possess a specific action on *T. brucei*.

Their work was continued by Moore, Nierenstein and Todd,* who concluded that the specific action of Atoxyl on trypanosomes can not only be explained on the assumption of a slow breakdown of Atoxyl into an inorganic arsenical and aniline ion, but is also 'due to direct and specific action of a complex organic ion containing both the aniline and arsenical groups.'†

Mesnil and Brimont‡ explain the action of Atoxyl on trypanosomes in a similar way. Their conclusions are based on the observation of a specific resistance of trypanosomes to drugs of the same type, but not to those of different types, such as parafuchsin on the one hand and Atoxyl and acetylated Atoxyl on the other hand, a fact which proves 'que l'atoxyl n'agit pas uniquement comme arsenical, mais en vertu d'un ion complexe.'

Uhlenhuth and Woithe§ incline to this view, as the symptoms in experimental animals caused through toxic doses of Atoxyl, are not those of either aniline or arsenic poisoning. They 'beziehen diese ganz besonders gearteten nervösen Symptome auf den ganzen Atomcomplex des Atoxyls.' The Atoxyl as such has a specific action on the cells of the organism, after they have undergone a change in some way or other under the influence of the parasites. This hypothetical action on the cells results in an increased formation of specific antibodies, which still continues even after the Atoxyl has been eliminated from the organism. These antibodies destroy the greater number of the parasites and prevent the few surviving trypanosomes from multiplying and injuring the host.¶

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*(*a*) Moore, Nierenstein and Todd. Bio-chemical Journal, Vol. II, No. 4-5, 1907.

(*b*) Moore, Nierenstein and Todd. Annals of Tropical Medicine and Parasitology, Vol. II, No. 4, 1909.

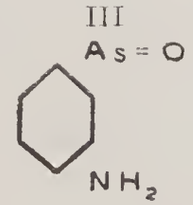
† Moore, Nierenstein and Todd. Bio-chemical Journal, Vol. II, No. 4-5, p. 322, 1907.

‡ Mesnil and Brimont, Comptes Rendus de la Soc. de Biol., Tome LXIV, 1908, p. 639.

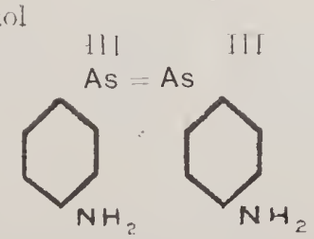
§ Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Band XXIX, 1908. Reprint p. 34.

¶ Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Bd. XXVII, 2. Heft, 1907.

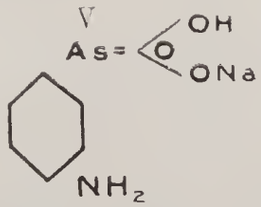
On the other hand, Ehrlich* and his collaborators express quite a different view of the action of Atoxyl. They assume that Atoxyl changes in the organism into a compound of a more toxic, and, therefore, more trypanocidal character, and consider that the pentavalent arsenic atom in Atoxyl becomes reduced in the organism to a trivalent atom, which form they regard as the active trypanocidal arsenic. Their view is based on the observation that p-amino-phenyl-arsen-oxide



and di-amino-arseno-benzol



(both reduction products of Atoxyl) have a marked trypanocidal action *in vitro*, whereas Atoxyl



as has been shown by a great number of observers, does not influence the parasites *in vitro*.

They believe, therefore, that the Atoxyl molecule is reduced in the organism, and products of a similar type, such as p-amino-phenyl-arsen-oxide and di-amino-arseno-benzol, are formed. Röhl,† Ehrlich's co-worker, goes so far as to state that the action of Atoxyl is only due to the formation of p-amino-phenyl-arsen-oxide.

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* (*a*) Ehrlich Verhandlungen der deutschen dermatologischen Gesellschaft, X. Congress, 1908.

(*b*) Ehrlich. Berichte der deutschen chemischen Gesellschaft, Jg. XLII, Heft I, 1909.

† Röhl. Berl. klin. Wochenschrift, No. 11, 1909.

They assume in the protoplasm of the trypanosome the existence of a special chemical complex (Arseno-receptor) which possesses a great affinity for trivalent arsenic.

Ehrlich's theory led to Levaditi's and Yamanouchi's* studies on the mechanism of the action of Atoxyl. By the action of an emulsion of animal tissue, especially liver, they were able to transform Atoxyl, which is inactive *in vitro*, into a trypanocidal compound 'trypanotoxyl,' which they regard as a combination of reduced Atoxyl with protein, a 'toxalbumine arséniée' of a thermolabile character. Levaditi compares this 'toxalbumine arséniée' with a haemolysin; the arsenic plays the part of a complement; the protein nucleus, the rôle of an amboceptor. The arsenic, therefore, cannot attach itself to the trypanosomes without the intercurrence of the protein nucleus of the toxalbumine arséniée.†

In a somewhat different way Friedberger‡ succeeded in transforming Atoxyl into a trypanocidal compound *in vitro* by adding thioglycolic acid to a solution of Atoxyl. The leading idea in his experiments was Heffter's theory of the reductive properties of the organism; according to the latter the protein 'reductases' are not to be considered living ferments, but the reduction is brought about by sulphhydryl or SH groups which also are present in this glycolic acid.

Our experiments,§ however, have brought forward very little evidence for the supposition that a reduction process in the organism plays a prominent rôle in the action of Atoxyl on trypanosomes. As a matter of fact, all our results tend to show that the transformation of Atoxyl into a powerful trypanocide *in vitro* and *in vivo* is mainly

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* (*a*) Levaditi et Yamanouchi. Comptes Rendus de la Soc. de Biol. Tome LXV, p. 23. 1908.

(*b*) Levaditi, Brimont et Yamanouchi. Comptes Rendus de la Soc. de Biol., Tome LXV, p. 25, 1908.

(*c*) Levaditi. Bull. de la Soc. de Path. exotique, Tome II, p. 45, 1909.

(*d*) Levaditi. Comptes Rendus de la Soc. de Biol., Tome LXVI, p. 33, 1909.

(*e*) Levaditi. Comptes Rendus de la Soc. de Biol., Tome LXVI, p. 492, 1909.

† Röhl in a more recent publication (Zeitschrift f. Immunitätsforschung, Vol. II, p. 496, 1909), points out that Levaditi does not furnish any conclusive proof for his conception of the formation of trypanatoxyl in the organism, and asserts that the trypanocidal effect of Atoxyl *in vivo* is only due to the formation of p-amino-phenyl arsenoxide.

‡ Friedberger. Berl. klin. Wochenschrift No. 38, 1908.

§ Breinl and Nierenstein. Zeit f. Immunitätsforschung, etc., Vol. I, p. 620.

due to an oxidation process. A reduction takes place in the intestines only, and is probably of secondary importance.

In our experiments we adopted Levaditi's and Yamanouchi's technique; after repeated failures to get any changes in an Atoxyl solution when shaken up for twenty-four hours at room temperature with an emulsion of different organs (liver, brain, etc.), 10 c.c. of a 4 per cent., 2 per cent., and 0·2 per cent. solution of Atoxyl in physiological saline were mixed with an equal amount of liver emulsion and kept at a temperature of 37° C. After two hours the effect of this mixture on trypanosomes was tried in a coverslip preparation. Only three experiments out of seven confirmed Levaditi's and Yamanouchi's observation that the parasites became immobilised and after a time destroyed.

After dialysing an Atoxyl-liver mixture which had been found to have a marked trypanocidal action in a coverslip preparation and concentrating the dialysate to the original volume, when it became again isotonic, we were able to observe that the dialysate acted upon the parasites in the same way as the original Atoxyl-liver mixture; the slightly acidified dialysate gave a distinct precipitate of arsenic sulphide when acted upon by H_2S.

We found that only in those cases in which, on mixing Atoxyl solution with liver emulsion, inorganic arsenic was present had the mixture a decided and rapid trypanocidal effect in vitro. On the other hand, in no case in which arsenic could not be detected, could any influence on the parasites be observed.

Similar results with regard to the appearance in the solution of inorganic arsenic were obtained when pure liver oxydase or oxydase of black tea was added in a sufficient quantity to 1 per cent. solution of Atoxyl, or when H_2O_2 was mixed with a 4 per cent. or 2 per cent. solution of Atoxyl and exposed for some time to a temperature of 37° C.

If reductase prepared from yeast was aded to Atoxyl solutions of different strengths, in some instances inorganic arsenic was set free from the Atoxyl together with aniline. This observation offered an explanation of the presence of aniline in the faeces, previously recorded.*

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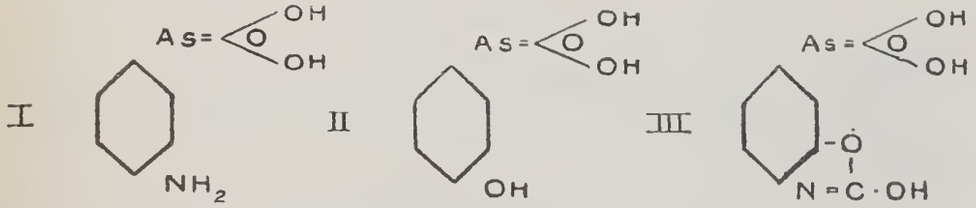
* Nierenstein. Annals of Tropical Medicine and Parasitology, Vol. II, No. 4, 1909.

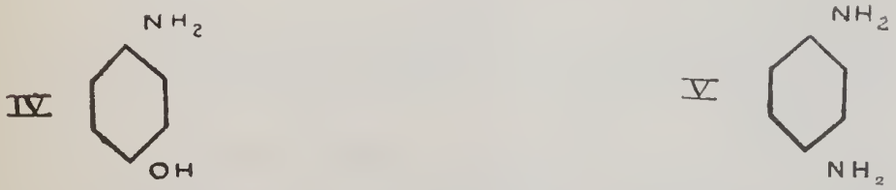
In order to imitate as far as possible, *in vitro*, the changes which Atoxyl undergoes in the organism, an 'Atoxyl-serum' was prepared by mixing a solution of Atoxyl with serum and dialysing out the excess of Atoxyl which had not combined with the serum proteins through the amino group. On the addition of liver emulsion or H_2O_2 to this 'Atoxyl-serum' inorganic arsenic was set free.

Nierenstein's* research on the mode of elimination of Atoxyl in the urine confirms our conception that Atoxyl mainly undergoes oxidation in the organism. After injecting the drug into a horse, it was recovered in the urine in the form of p-amino-phenyl-arsenic acid (Formula I), p-oxy-phenyl-arsenic acid (Formula II), and oxy-carb-amino-phenyl arsenic acid (Formula III).



It is obvious that the formation of the compounds II and III from I (the mother substance of Atoxyl) can only be explained through an oxidation process in the organism.

The formation of p-oxy-phenyl-arsenic acid (Formula II) from p-amino-phenyl-arsenic acid is brought about through a replacement of the amino group by an HO group, and is similar to the formation of p-amino-phenol (Formula IV) after injection of p-amino-aniline (Formula V).



This oxidation process enables the organism to eliminate aniline and Atoxyl in a less toxic form as the sulphuric or glycuronic derivatives.

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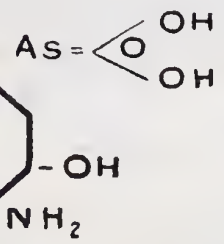
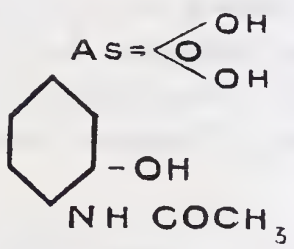
* Nierenstein. Zeit. f. Immunitätsforschung, Bd. II, No. 4, 1909.

The formation of oxy-carb-amino-phenyl-arsenic acid can be explained in the following way. The organism introduces on oxidation an OH group in the o-position to the amido group, and thus forms p-amino m-oxy-phenyl-arsenic acid (Formula VI). This oxidation is then followed (based on the analogy of toluidine) by an intermediate acetylation of p-amino- m-oxy-phenyl-arsenic acid in the organism (Formula VII). The acetyl chain is oxidized, and forms oxy-carbonyl arsenic acid (Formula III).

VI



VII



On considering the foregoing experiments and results, it becomes possible to divide the action of Atoxyl in the organism into four phases.

I. After injection of Atoxyl, a comparatively small amount combines through the amino group with the serum proteins and forms 'Atoxyl-serum'; the greater part is secreted in the urine, partly unchanged as p-amino-phenyl-arsenic acid, partly oxidised in the form of p-oxy-phenyl-arsenic acid and oxycarb-amino-phenyl-arsenic acid, and partly as free inorganic arsenic.

II. From the Atoxyl serum, arsenic is set free through an oxidation process, caused by the oxidative ferments present, and probably also by the trypanosomes, whereby the aromatic nucleus is destroyed.

III. At the same time a reduction process takes place in the intestines, whereby the Atoxyl molecule is reduced to aniline and arsenious acid.

IV. The arsenic which is formed through oxidation acts *in statu nascendi* on the trypanosomes.

As has been pointed out before, the action of Atoxyl can be compared to that of a dye.* The amino group plays the rôle of a

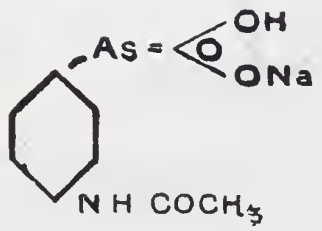
* Nierenstein. Annals of Tropical Medicine and Parasitology, Vol. II, No. 3, p. 249, 1908.

chromogenetic group, the arsenical radical acts on the parasites in the same way as a chromophoric group on a cotton fibre, whereas the serum plays the rôle of a mordant.

B. *Derivatives of Atoxyl.*

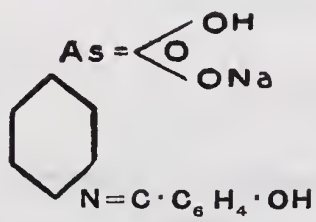
In order to ascertain whether certain derivatives of Atoxyl have a superior therapeutical value in trypanosome infections, a great number of compounds were experimented with.

1. Acetylated Atoxyl.



Acetylated Atoxyl was first prepared by Ehrlich and Bertheim. It was used by C. Browning, by Moore, Nierenstein and Todd, and by Breinl. It proved to be less toxic for animals very susceptible to Atoxyl, such as dogs, which, as Ehrlich* points out, is not the case in horses and guinea-pigs. The only difference between it and Atoxyl, consists in the partial acetylation of the amino group, which change apparently reduces the toxic effect of the aniline nucleus in the Atoxyl. Uhlenhuth and Woithe† believe that the acetyl group prevents the rapid discharge of arsenic, a conception which is improbable. It has been pointed out by Nierenstein, that part of Atoxyl undergoes acetylation in the organism, which observation probably explains the less toxic effect of acetylated Atoxyl.

2. Salicyli-Atoxyl.



This compound was prepared with the view of introducing Atoxyl *in statu nascendi* into the organism, as it might be expected that on

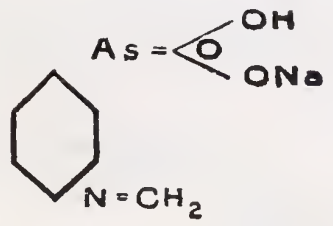
* Ehrlich. Berl. klin. Wochenschrift, No. 9-12, 1907. Reprint, p. 22.

† Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Band XXIX, 1908. Reprint, page 42.

hydrolysis, p-amino-phenyl-arsenic acid and salicylic acid would be formed. The salicylic acid might then act as an internal disinfectant.

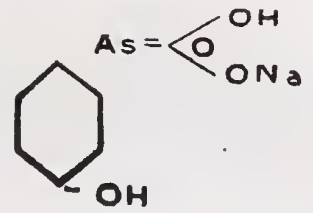
A few experiments on rats infected with *T. brucei* showed that doses of as much as 0·025 gm. per rat of about 170 gm. did not cause a disappearance of the parasites from the blood.

3. Formylo-Atoxyl.



Formylo-Atoxyl brought about in rats infected with *T. evansi* a temporary disappearance of the parasites after two to three injections of 0·025 gm.; the trypanosome, however, reappeared in the course of a few days. The drug caused abscesses at the site of injection.

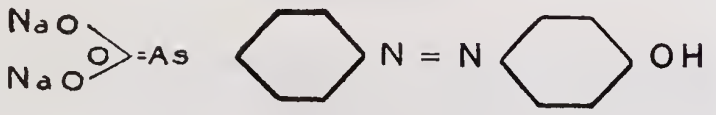
4. Sodium p-hydroxy-phenyl-arsenate.



This compound was found to have no effect whatever on the parasites, a fact which corresponds with the view that the presence of the amino group is essential for a trypanocidal action.

5. Di-sodium Azobenzene 4-arsenate

and Di-sodium 4-oxy Azobenzene 4-arsenate.



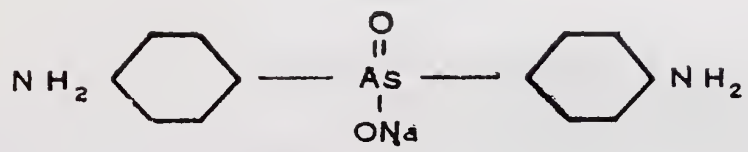
From a theoretical point of view, it might be expected that after injection both compounds would on reduction in the organism break

up into free p-amino-phenyl-arsenic acid, and that the latter, together with an amino group *in statu nascendi*, might have a greater affinity for the serum proteins and combine with them to a larger extent. Experiments, however, have shown that this process does not take place in the organism, as even large doses had no appreciable effect on rats infected with trypanosomes.

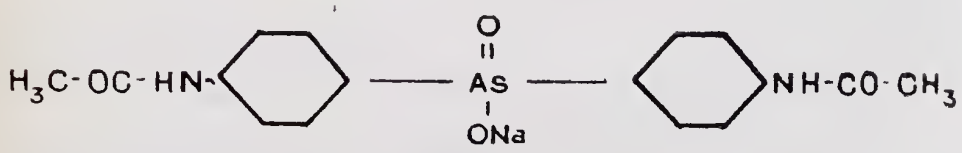
6. Tetra sodium phenazine 4-arsenate.

The destructive effect of phosphine, a colouring matter of the safranine type,* on *Paramaecium* is very marked, and is not surpassed by any other substance. We, therefore, hoped that on combining an arsenic molecule with a phenacine, a colouring matter of the same type, the result would be a compound of increased trypanocidal value in comparison with Atoxyl. Experiments, however, proved that this compound did not affect trypanosomes. Reference may here be made to the work of Mannaberg,† who finds that phosphines have no beneficial effect in the treatment of Malaria.

7. Sodium di-p-amino-phenyl-arsenate



and Sodium di-p-acetyl-amino-phenyl-arsenate.



These compounds, even if used in the largest doses which could be administered, were of no value in the treatment of rats infected with *T. equiperdum*.

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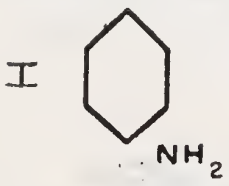
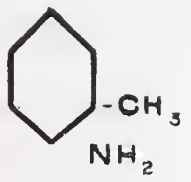
* Frankel M. Die Arzneimittelsynthese auf Grundlage der Beziehg. zwischen chemischen Aufbau und Wirkung. 2. Aufl., 1906, p. 206.

† Mannaberg. Arch. f. klin. Medizin, Bd. 59, p. 185.

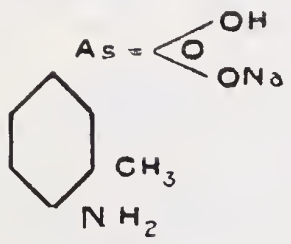
C. Orsudan

Experiments on a larger scale have been carried out with substances closely allied to Atoxyl, but having, instead of an aniline nucleus (Formula I), a toluidine nucleus (Formula II).

I 

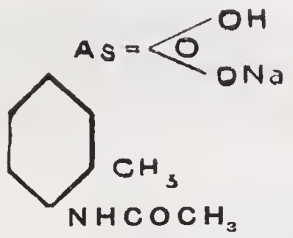
II 

I. Sodium 3-methyl 4-amino-phenyl-arsenate (Kharsin)



and its acetyl derivative.

Sodium 3-methyl 4-acetyl-amino-phenyl-arsenate (Orsudan).



After a few preliminary experiments the acetylated compound Orsudan was found to be the less toxic of the two, and most of the treatment experiments were carried out with it. It was a remarkable fact that Orsudan did not have any effect on rats or on one donkey infected with *T. brucei*, although two strains of this parasite, of different origin, were used. On the other hand, it acted promptly on *T. equiperdum* and *T. gambiense*.

In rats infected with *T. equiperdum*, parasites disappeared after the first injection of 0·025 gm. of the drug, but usually recurrences were observed after a varying period if treatment was discontinued. Only a few rats lived as long as eight months without reappearance of parasites, but these also succumbed to intercurrent diseases. Two rats showed relapses after a very prolonged period, one, 126 days after the last injection of Orsudan (0·075 gm. had been administered),

the other, 105 days after discontinuance of treatment (0·06 gm. of Orsudan was administered in three injections).

Out of twelve rats infected with *T. gambiense* one lived for 165 days, one for 115 days (both dying without having shown parasites in the blood), and one had a relapse fifty days after the last injection.

Guinea-pigs infected with *T. gambiense* stood Orsudan much better than Atoxyl. The parasites always disappeared from the blood after the first injection of 0·025 gm. When the treatment was discontinued, relapses set in after a varying interval. If treatment was continued the intervals between the relapses became shorter and shorter, until a point was reached when even repeated injections had no effect on the parasites, and the animal succumbed to the trypanosome infection. If the parasites were subinoculated into guinea-pigs, one injection of Orsudan was sufficient to drive out the parasites.

Three donkeys, infected with *T. equiperdum*, were treated with Orsudan. Two died after two injections (each of one gramme) after typical symptoms of arsenic poisoning lasting three days. The internal organs showed fatty degeneration of the liver and kidneys. One donkey died of Trypanosomiasis 227 days later. It received, during this period, nine injections, each of one gramme of Orsudan; parasites in small numbers were occasionally seen in the blood.

Four monkeys infected with *T. gambiense*, after the infection was well established, were treated with Orsudan. One *Cercopithecus callitrichus* died with all the symptoms of typical arsenic poisoning, after having received 0·2 gm. in two injections. One *Cercopithecus mona* received 0·2 gm. in two injections, and had a relapse forty-nine days after the last injection. The treatment was repeated with 0·1 gm. of Orsudan. Although symptoms of arsenic poisoning set in a few days afterwards, they passed off in a week, and the animal lived for 254 days in good health without showing any parasites. On reinoculation of *T. gambiense* it succumbed to the infection.

One *Cercopithecus callitrichus* received 0·2 gm. of Orsudan in two injections. Three days afterwards severe symptoms of arsenic poisoning set in, which, however, passed off in a week's time. Twenty-five days afterwards treatment was resumed with one injection of 0·1 gm. Orsudan. Two days afterwards symptoms of arsenic poisoning commenced; four days later failure of eyesight was noticed,

and within three days the animal was totally blind. As no recovery of vision took place, the animal was killed with the intention of examining the pathological lesions of the eye.

One *Macacus rhesus* was inoculated with *T. gambiense*. Treatment was begun after the second natural relapse. After one injection of 0·1 gm. of Orsudan the parasites promptly disappeared. Thirty-five days afterwards a second injection of 0·1 gm. of Orsudan was given. During the following day symptoms of severe arsenic poisoning set in, and the animal became totally blind six days after the injection. This monkey is still alive thirteen months after the inoculation. He is absolutely blind, and no visible changes can be noticed in his eyes.

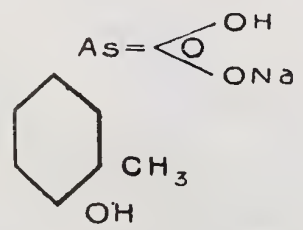
The foregoing experiments lead to the conclusion that Orsudan as a trypanocide is, for *T. gambiense* and *T. equiperdum* in experimental animals, nearly equal to that of Atoxyl.

As with Atoxyl, only in a very small percentage of our experiments have we been able to prevent relapses. On the other hand, Orsudan is certainly very toxic indeed, as our experiments in monkeys show; out of four animals treated, two cases of blindness and one death from typical arsenic poisoning occurred. The higher toxicity, when compared with Atoxyl, may be explained on the hypothesis that Orsudan combines to a larger extent with the tissue than Atoxyl, owing to the fact that CH_3 group is oxidised to COOH, and, therefore, more arsenic combines and is afterwards set free in the organism.*

In a few of our experiments on rats, haemoglobinuria, the result of haemolysis, was noticed. This may be due to the well-known fact that toluidine derivatives are more powerful haemolytic agents than aniline derivatives.

D. *Derivatives of Orsudan.*

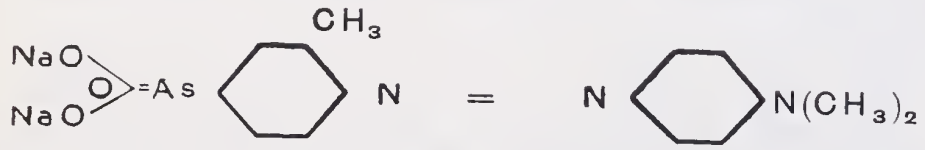
1. Sodium 3-methyl 4-hydroxy-phenyl-arsenate.



* Lately we have had an opportunity of testing a new sample of Orsudan. Two successive injections of 0·2 gr. into a monkey did not give rise to any symptoms of arsenical poisoning.

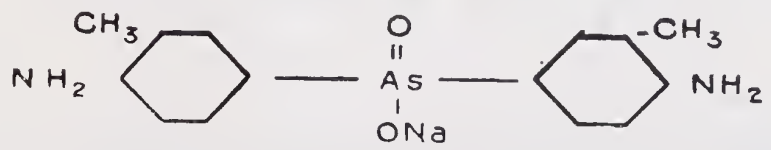
The replacement of the amino group by an hydroxyl group changes the character of the compound in the case of Orsudan as it does in Atoxyl. It is extremely toxic and showed only a very slight action on the parasites, and only when injected in so large doses as 0·05 gm., in which case it killed the animal in a few hours.

2. Di-sodium-4-di-methyl-amino 2-methyl-azo-benzene 4-arsenate.

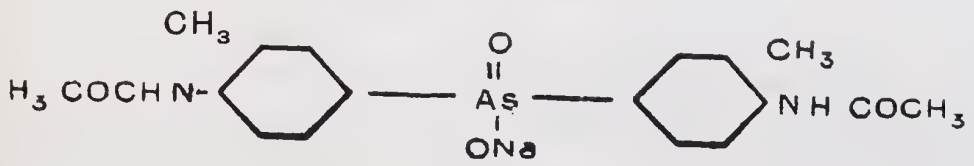


was found to have a slight effect on trypanosomes. They decreased in number, but the rats usually died within a few days. When compared with the corresponding Atoxyl derivative No. 5, p. 401, it is seen that although the combining amino group is saturated through diazotising, in this case the CH_3 group most probably gets oxidised to a COOH group, through which a combination with the proteids takes place.

3. Sodium di-(3-methyl 4-amino-phenyl) arsenate



and Sodium di-(3-methyl 4-acetyl-amino-phenyl) arsenate

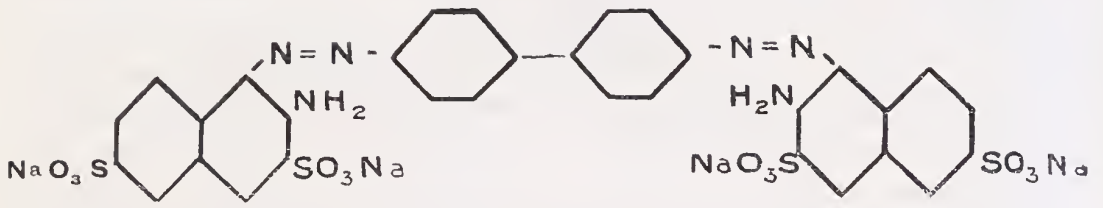


had, in the same way as the corresponding Atoxyl derivative, on injection, no effect on rats infected with *T. equiperdum*.

COLOURING MATTERS

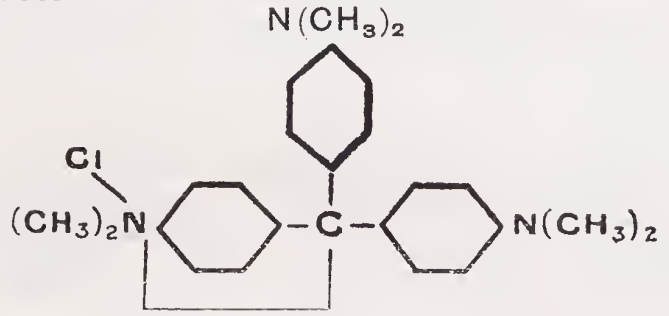
Since Ehrlich and Shiga's discovery that trypanred has a pronounced trypanocidal action, numerous colouring matters of different chemical constitution have been tried in experimental Trypanosomiasis with a more or less therapeutical success.

The preparation of trypanred by Ehrlich and Shiga (Formula I)



has led to the discovery of afridol blue by Nicolle and Mesnil (Formula II).

Afterwards Ehrlich introduced parafuchsin (Formula III) and tryparosan, a chlorinated parafuchsin, in the treatment of experimental trypanosomiasis.



The value of all these colouring matters has been put to a severe test in this laboratory, but with discouraging results. Thomas and Breinl* in 1905 remarked in reference to trypanred, 'that we cannot claim to have cured any animal infected with the parasites of surra, ngana and dourine. The disease, especially in rats and mice, may be greatly prolonged, but the animals eventually die.' Moore, Nierenstein and Todd† came to a similar conclusion 'that trypanroth was not always able to prevent an early death from the disease.'

Afridol blue and parafuchsin, phosphines and numerous other colouring matters, had in our hands only a slight effect, or no effect at all, on the parasites. We may say that hardly any work with these

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* Thomas and Breinl. Liverpool School of Tropical Medicine, Memoir XVI, p. 51, 1905.

† Moore, Nierenstein and Todd. Annals of Trop. Med. and Parasitol., Vol. II, No. 4, p. 285.

colouring matters was carried out on mice, a fact which may account for our inability to confirm Ehrlich's experiments.

Ehrlich* suggested that parafuchsin may act as a prophylactic for trypanosome infection. He was successful in preventing infection in mice after feeding them with parafuchsin and then inoculating the parasites.

We attempted to confirm these experiments on large animals. Two large horses were fed on parafuchsin. One of them received fifteen grammes daily by mouth, for thirty days and died after having shown toxic symptoms from the parafuchsin. A second horse received fifteen grammes by mouth for forty-eight days. On inoculation, it became infected in the same way as an untreated animal.

In our opinion, it is not necessary to compare the action of colouring matters on trypanosomes with the action of Atoxyl. Whereas in Atoxyl the amino group, according to our experience, effects a combination between the proteins and the Atoxyl molecule, and the specific action on the parasites is due only to the liberated arsenic; in colouring matters the trypanocidal effect is most probably due to the amino group. Mesnil and Nicolle† already expressed the view that the therapeutical effect may be due to the presence of nitrogen.

Moore, Nierenstein and Todd‡ came to the conclusion that the NH_2 group is the active trypanocidal radical, for which they suggest the name 'trypanophobe group.' This conception corresponds to the observations of Loew and Bokorny§ on the influence of compounds containing amino groups upon the multiplication of algae. They found that with an increase of the number of amino groups in compounds of the type of urea and uric acid, the noxious influence upon the plant increases.' They explain the observation in this way: that the protoplasm contains a great number of labile aldehyde and amino groups which combine alternately with the amino and aldehyde

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* Ehrlich. Berl. klin. Wochenschrift, 1907. Reprint, p. 31.

† Mesnil and Nicolle. Annales de l'Instit. Pasteur, XX, p. 417, 513, 1906.

‡ Moore, Nierenstein and Todd. Annals of Tropical Medicine and Parasitology, Vol. II, No. 4, 1909, p. 271.

§ Loew und Bokorny. Jour. f. prakt. Chemie, Bd. 36, p. 272. (Compare, Fränkel, *loc. cit.*, p. 29.)

groups of the drug. This theory may be applied to the action of aniline dyes upon trypanosomes. In this case the labile aldehyde groups of the protoplasm of the trypanosome may combine with the amino group of the drug. Gabritschewsky* explains in a similar way the slight neutralising action of fuchsin and vesuvin if brought in contact with toxins.

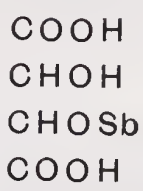
ANTIMONY COMPOUNDS

Plimmer and Thompson† were the first to introduce antimony in the treatment of experimental Trypanosomiasis. Their results were encouraging. These good results, however, have not been fully confirmed by other workers. Plimmer and Thompson used sodium, potassium and lithium salts of antimonyl tartrate. On considering the chemical constitution of these compounds, it is apparent that they were not, in fact, dealing with an organic compound of Antimony, but only with an alcoholate of tartaric acid (Formula I) and antimonic oxide.

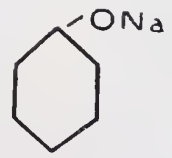
Antimonyl tartrate has the following constitution (Formula II). The antimonic oxide in this case is not in an aromatic chain as the arsenic in Atoxyl, but forms only an alcoholic salt similar to sodium in a phenolate (Formula III).

I

II



III



The action of sodium antimonyl tartrate is an antimonic action pure and simple. On injection, dissociation of the compound into sodium, tartaric acid, and antimonic oxide takes place, the latter of which acts destructively on the parasites. A similar process takes place on mixing the compound with infected blood, even in the dilution of 1 : 40,000 the trypanosomes are immobilized in a few minutes.‡

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* Gabritschewsky. Arch. Internationales de Pharmakodynamie et de Therapie, Vol. VII, Fascl. 1-2, p. 115.

† Plimmer and Thompson. Proc. of the Roy. Soc. B., Vol. 80, 1907.

‡ Broden et Rodhain. Travaux du laboratoire médical de Leopoldville, III, 1909, p. 48.

This rapid liberation of antimonic oxide, which, as is well known, is very slowly resorbed by the tissues and causes great irritation, may be the cause of abscess formation at the site of injection. This disadvantage of abscess formation was overcome by introducing an organic antimony compound of a similar type to Atoxyl into the therapeutics of Trypanosomiasis,* the antimony radical being linked on to the aromatic nucleus. From the three isomeric arylstibinic acids only the para derivative gave satisfactory results. The meta compound was far less stable, as it partly decomposed on standing into antimonic oxide and aniline. The ease with which this compound decomposes may be held responsible for the occurrence of abscesses after injection. The antimony is probably too quickly liberated and not sufficiently quickly resorbed.

The mechanism of the action of the p-amino-phenyl-stibinic acid is similar to that of p-amino-phenyl-arsenic acid (Atoxyl). It is remarkable on comparing the action of sodium antimonyl tartrate and of sodium p-amino-phenyl-stibinic acid, that whereas the first named compound effects a disappearance of the parasites within one hour, the latter compound produces the same effect only after the lapse of 15-19 hours. The effect stands in direct proportion to the rate at which antimony is set free from the drugs.

RESISTANCE

Thomas and Breinl† observed in their work on Atoxyl in experimental trypanosomiasis, that in a horse infected with *T. evansi*, the parasites disappeared from the blood after administration of Atoxyl. In spite of continuation of the treatment, parasites were seen again, and 'were twenty-five to a field on the fourth day after the dose in the last week but one. Two doses per week were therefore commenced; this kept the number of parasites down, so that one to five fields was the highest reached.'

Similar observations were made by Mesnil and Nicolle,‡ using benzidine colours for the treatment of experimental trypanosomiasis.

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* Breinl and Nierenstein. Annals of Tropical Medicine and Parasitology, Vol. II, No. 5, 1909.

† Thomas and Breinl. Liverpool School of Tropical Medicine, Memoir XVI, 1905, p. 65.

‡ Mesnil and Nicolle. Annales de l'Instit. Pasteur, No. XX, p. 528, 1906.

They found that after continuation of the treatment for some time the drug had no effect upon the parasites.

Franke* found, 'working with mice infected with Ngana and treated with parafuchsin, that when the recurrences ceased to respond to the treatment, inoculation into fresh animals gave rise to an infection which was from the very beginning uninfluenced by parafuchsin, administered either by feeding or by injection.'

Browning† then observed a similar behaviour of certain trypanosome strains against Atoxyl.

It was, however, Ehrlich who first recognised the general importance of this phenomenon, and drew attention to the fact that one can produce strains resistant to all types of trypanocides. He had at his disposal strains resistant against—(1) Atoxyl, (2) Trypan-red, (3) Trypanblue, (4) Atoxyl and trypanblue, (5) Arseno-phenyl-glycine, (6) Tartar emetic.‡

Ehrlich was further able to prove that this resistance may be preserved throughout many passages in mice, and he regards this feature as 'einen schönen Beweis für die Vererbung erworbener Eigenschaft.' However, in the case of one strain (Atoxyl strain, No. 1), which kept resistant for six months (67 passages), this acquired character was lost after seven and three-quarter months (87 passages). A striking characteristic of the resistance is its specific nature, i.e., if a strain has become resistant to parafuchsin it is resistant as well against all colouring matters of the tri-phenyl-methan

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* Quoted by C. H. Browning. Journal of Path. and Bacteriology, Vol. XII, 1908, p. 176.

† Ehrlich. Berl. klin. Wochenschrift, No. 9-12, 1907. Reprint, p. 22.

‡ Strains of trypanosomes resistant to tartar emetic have also been obtained by Mesnil and Brimont, by Plimmer and Bateman; and a strain of *T. brucei* resistant against p-amino-phenyl-stibinic acid has been obtained by us. This resistance, however, was not transmissible on subinoculation. Mesnil and Brimont succeeded in obtaining a permanent antimony resistant strain by treating an Atoxyl-resistant strain with antimony. At first the parasites were influenced by tartar emetic, but after the fifth injection they became resistant, a resistance which persisted for seventy-six passages.

A similar relative resistance was observed by us when treating guinea-pigs infected with *T. gambiense* with Orsudan. After six to seven injections the drug had lost its influence on the parasites.

Uhlenhuth, Hübner and Woithe, working with *T. equiperdum*, were not able to confirm entirely Ehrlich's observations. They were only able to produce a resistance in one animal after a certain number of relapses. On subinoculation, Atoxyl influenced the parasites in a normal way. Therefore, they regard it as a relative resistance, distinguishing it from an absolute resistance in Ehrlich's sense of the term.

group, though not resistant against Diazo-colouring matters, as trypanred, and arsenic compounds and *vice versâ*. Mesnil's observation contradicts to a certain extent this statement, as a strain resistant against Atoxyl was found to be influenced by Orsudan.

Ehrlich* explains this complex phenomenon on the assumption that the avidity of the arseno-receptors of the trypanosomes for arsenyl has diminished to such an extent, that the protoplasm of the trypanosomes has become unable to combine with the Atoxyl.

The fact that an Atoxyl-resistant strain is still influenced by arseno-phenyl-glycine seemingly contradicts his conception. The interpretation which Ehrlich gives, is that although the trypanosomes of an Atoxyl-resistant strain have lost the greater part of the avidity of their arseno-receptors for Atoxyl, yet enough still remains to permit of the action of arseno-phenyl-glycine being manifested.

In the hands of one of us an Atoxyl-resistant strain in mice, sent by Professor Ehrlich† early in 1907, was shown to lose its resistance against Atoxyl when inoculated into rats.

Independently, Mesnil and Brimont‡ came to a similar conclusion, 'que la race est resistante à l'Atoxyl dans un organisme donné.'

Breinl and Nierenstein,§ in their work with an Atoxyl-resistant strain of *T. brucei* in donkeys, were able to prove that the acquired resistance against Atoxyl only holds good for a given species of animal, and that this acquired character is retained for this given species even after prolonged passages through different animals in which parasites are not resistant. On subinoculation of the Atoxyl-resistant parasites into rats, only in the first generation was a slight resistance noticeable. In the second generation the parasites behaved in the same way as the normal control strain. Mesnil and Brimont¶ find that an Atoxyl-resistant strain in mice is still influenced to a certain degree by Asodyl, very slightly by trisulphide of arsenic. Whether arsenious acid still effects Atoxyl-resistant trypanosomes or

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* Ehrlich. Verhandlungen der deutschen dermatologischen Gesellschaft, X, Congress, 1908.

† Ehrlich. Jour. of the Roy. Inst. of Public Health, Vol. XX, No. 7, p. 391.

‡ Mesnil et Brimont. Comptes Rendus de la Soc. de Biol., Tome LXIV, 1908, p. 637.

§ Breinl and Nierenstein. Deutsche med. Wochenschrift, No. 27, 1908.

¶ Mesnil et Brimont. Annales de l'Instit. Pasteur, Tome XXII, p. 856, 1908.

not, they were unable to decide definitely. They confirm the above-mentioned observations of Breinl and Nierenstein, laying particular stress on the point that a slight resistance may still be noticed when Atoxyl-resistant trypanosomes are subinoculated into a different species, such as from mice into a dog or a guinea-pig.

Röhl* states that trypanosomes which are Atoxyl-resistant in mice are, on inoculation into rats, not absolutely resistant, though certainly so to some extent. He, however, does not explain whether this resistance was only noticeable in the first generation. Furthermore, he brings forward experimental evidence which tends to show that an Atoxyl-resistant strain in mice on subinoculation into rats is not affected by Atoxyl. However, as inoculation and injection were done simultaneously, in our opinion no definite conclusion can be formed.

We regard the resistance as an acquired immunity of the trypanosomes against the Atoxyl-serum, as we have previously pointed out. This is in accordance with our observation, confirmed by different observers, that the Atoxyl resistance only holds good for the one species in which it has been acquired. The trypanosomes have become tolerant only to the *one* Atoxyl-serum combination, as for example in the mouse, and are still influenced by the Atoxyl-serum of the rat.

A similar view has been expressed by Mesnil and Brimont.† In further experiments, however, with an Atoxyl-resistant strain in mice, sent to us by Professor Ehrlich, we observed that the resistance after subinoculation from mice into rats was still well marked in the latter animals even after three passages. This result, also obtained by Röhl, seemingly contradicts our previous observations that the resistance is confined to one animal species *only*. We agree with Röhl's opinion that the seemingly contradictory results concerning resistance can only be explained through the assumption of a gradual increase of the resistance, after numerous passages from a single animal, at first to animals of the same species, and later to animals of a different species.

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* Röhl. Berl. Klin. Wochenschrift, No. 11, 1909.

† Mesnil et Brimont. Annales de l'Instit. Pasteur, Tome XXII, p. 856, 1908.

THE REGULARITY OF THE TIME OF REAPPEARANCE OF PARASITES AFTER TREATMENT

It is a well-known fact that after the injection of a trypanocide in experimental trypanosomiasis the parasites disappear. If the treatment is then discontinued, the parasites usually reappear after a variable period. In our large experience with *T. gambiense* and *T. brucei* we have been able to observe that, to a certain extent, a regularity prevails in the interval between disappearance and reappearance. In infection with *T. gambiense* in rats and monkeys, this interval usually extends over fifty to sixty days after injection of Atoxyl or Orsudan and discontinuance of treatment. In infections with *T. brucei* in rats, guinea-pigs and dogs, on the other hand, only sixteen to twenty-five days elapse before reappearance. This is a very interesting observation, for which we are at present unable to account. It suggests that Atoxyl may be retained in the organism for such a period, and that only after its entire removal, the multiplication of the very few surviving parasites sets in anew. This discrepancy between the relapsing time after infection with *T. brucei* and *T. gambiense* may be due to a slower multiplication of the latter parasite in comparison with the former. In our therapeutical experiments with p- and m-amino-phenyl-stibinic acid the relapsing time did not generally show the same regularity.

It is possible, on the other hand, that the course of the relapses is determined by the life-history of the trypanosome. Breinl and his co-workers were able to work out a life-history of *T. gambiense* in the warm-blooded host, but after prolonged research were unable to observe any life cycle of *T. brucei*, and could only demonstrate multiplication by simple fission. This observation makes it doubtful if the regularity of relapsing time can be dependent upon a definite life cycle.

VALUE OF SUBINOCULATIONS

Particular stress has been attributed to the value of subinoculation of peripheral blood from an animal after treatment, in order to decide whether the animal has been cured from the disease. In our opinion, no conclusion concerning a definite cure can be drawn from subinoculation. If subinoculations are made shortly after injections of a

drug, even with large quantities of blood into susceptible animals, very rarely do parasites appear in the blood of the subinoculated animal. The result of subinoculations after a longer interval varies considerably. Even in the case of large animals infected with *T. brucei*, after treatment and temporary disappearance of the parasites, subinoculations are very frequently negative; sometimes, however, an infection occurs after a very prolonged incubation period.

Less reliable still are subinoculations from cases of Sleeping Sickness patients into rats and guinea-pigs.

The following is an illustration of this statement.

From a patient in a comparatively early stage of trypanosomiasis, at a time when parasites could be seen in very small numbers in the peripheral blood, subinoculations were made into a guinea-pig and two rats. The guinea-pig, although examined daily for six months, never became infected after injection of 2 c.c. of blood. Of the two rats, both showed parasites on very rare occasions, and then only in very scanty number (1 to 3 in each coverslip preparation). One showed parasites on 16 days out of 111 days, the other on 14 days out of 14 months; both died from intercurrent diseases. Although subinoculations upon rats were made from the original rats on days when parasites were observed, none of the subinoculated animals ever became infected.

WHEN CAN AN ANIMAL BE CONSIDERED TO BE CURED?

Many statements have been made concerning the efficiency of different drugs with regard to the effect of permanent cures in animals. One is naturally inclined to conclude, from a rapid and apparently complete destructive action of arsenic and antimony upon trypanosomes contained in the blood, that the organism is freed from the parasites. However, careful examination of the blood after treatment reveals the fact that only too often parasites reappear again, even after very prolonged periods. We were able with careful daily examination to observe relapses in rats infected with *T. brucei* and treated with Atoxyl after a negative period of 226 days; and in rats infected with *T. equiperdum* and treated with Orsudan as late as 105 and 126 days after discontinuation of the treatment.

Similar observations have been made by Uhlenhuth and Woithe with regard to a dog infected with *T. equiperdum*, subsequently treated with Atoxyl.

In animals infected with *T. gambiense*, the average relapsing time after discontinuation of treatment, as previously remarked, lies between 50 and 60 days; therefore, observation over a very prolonged period is necessary before considering an animal cured. Occasionally, even in an untreated monkey during an infection with *T. gambiense*, longer intervals may occur, during which parasites, in spite of careful daily examination, cannot be detected, and only intercurrent diseases which lower the vitality of the infected organism cause a reappearance of the parasites. Two of our infected monkeys succumbed to an intercurrent pneumonia and pleurisy. In the blood of the animals, although parasites had not been seen for 41 days, trypanosomes in scanty number reappeared on the day before death.

A third monkey, a very large *Cercopithecus callitrichus*, had seemingly recovered easily from an infection with *T. gambiense*. Its weight increased, the blood count was normal, and we were inclined to assume a natural cure in this animal. Subinoculations made upon rats proved negative. After a negative interval of 74 days the animal suddenly became markedly ill. This was due to an abscess of the gum, which spread in a very short time along the upper jaw and led to the death of the animal at the end of three days. On the day previous to death, trypanosomes were seen in the peripheral blood in very scanty number (1 to 3 coverslip preparations). At death only one parasite could be found in three coverslip preparations. Subinoculations into two guinea-pigs and two rats were made after death with fairly large quantities of blood. One guinea-pig inoculated intraperitoneally succumbed to a purulent peritonitis; the second guinea-pig, inoculated subcutaneously, developed an abscess at the site of inoculation, but became infected after an incubation period of 15 days. Both rats, inoculated subcutaneously, developed a typical infection of *T. gambiense*, and succumbed in due time to the disease.

These observations, together with many others made during our experimental work, point to the assumption that the general condition of experimental animals influences to a large extent the results obtained in therapeutical experiments.